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Filed : June 25, 2003

REMARKS

Claims 1 and 22 are amended. No new matter has been introduced herewith. The following addresses the substance of the Office Action.

Rejection under 35 U.S.C 112, first paragraph

Claims 1 and 15 are rejected as failing to comply with the enablement requirement. The Patent Office has taken the position that there is no enabling disclosure for the claimed method of evaluating cell activation by evaluating the phosphorylation state of named proteins from the list of Table 1 in the Specification. In particular, the Examiner has alleged that the specification is not enabling with regards to CDK8 and PAK6.

CDK8

The Examiner has cited Hoeppner, stating that CDK8 can not be phosphorylated at a particular site known to be involved in the activation of other CDKs. It is known that other members of the CDK family can be activated by phosphorylation at the position corresponding to T160 in human CDK2. However, CDK8 can not be phosphorylated at this position since it is occupied by aspartate. Hoeppner et al. state that it is uncertain whether this aspartate residue mimics a phosphothreonine residue to activate the protein.

While it is true that CDK8 activity is not regulated by phosphorylation at the position analogous to T160 in human CDK2, the scientific literature suggests that CDK8 may be regulated by phosphorylation at other site(s). For example, Tassan et al. acknowledges the absence of a threonine phosphorylation site at position Thr-161, but teaches that CDK8 of human (K35) and *S. cerevisiae* (SRB10) both contain phosphorylatable residues corresponding to Thr-14 and Tyr-15 in subdomain I (equivalent to Thr-31 and Tyr 32 of human CDK8) (Tassan et al. 1995 *Proc Natl Acad Sci USA* 92:8871-8875, see page 8874, column 2, line 16 to page 8875, column 1, line 9 and Fig. 4 on page 8874 showing the potential phosphorylation site within a GXGXXG motif). Tassan et al. state at page 8875, first column, lines 5-7: "Hence, K35 [a.k.a., CD8] and SRB10 might be negatively regulated via phosphorylation of residues within the GXGXXG motif". Thus, the activation state (*i.e.*, lack of negative regulation) of cells could potentially be determined by quantifying the level of CDK8 phosphorylation.

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CDK8 is a Substrate for Protein Kinase A Phosphorylation

The attached Declaration under 37 C.F.R. § 1.132 by Dr. Jose Remacle shows that human CDK8 is a substrate for PKA. **Appendix A** is a copy of Dr. Remacle's curriculum vitae.

To establish that CDK8 is a substrate for protein kinase A (PKA) phosphorylation, the Applicants incubated recombinant human CDK8 protein with PKA catalytic subunit in the presence of radioactive [$\gamma^{32}\text{P}$]-ATP. Reaction products were analyzed by gel electrophoresis under denaturing conditions and the resolved products were analyzed by phosphorimaging. Referring to Figure 1 of Dr. Remacle's declaration, two radioactive bands were detected with apparent molecular weights slightly higher than the unlabelled, native protein, which migrates with an apparent molecular weight of 59 KDa. (Note: The applicants would be happy to send a hard copy of Figure 1 if the copy filed electronically is insufficiently clear). The two bands of radiolabeled CDK8 protein are highlighted by two arrows just above the 64 KDa marker. An increase in molecular weight can be explained by the attachment of one or more negatively charged phosphate residues to the CDK8 protein. The conclusion is that CDK8 is a substrate for PKA.

Phosphorylation Sites in Human, Mouse and *Drosophila* CKD8

The NetPhos 2.0 server is run by the Center for Biological Sequence Analysis on the internet at <http://www.cbs.dtu.dk/services/NetPhos/>. The program predicts serine, threonine and tyrosine phosphorylation sites in eukaryotic proteins. Referring to the attached **Appendix B** entitled "NetPhos 2.0 Server-prediction results, starting on the bottom of page 2 through page 4, human CDK8 is potentially phosphorylated at 13 serine residues, 9 threonine residues and 4 tyrosine residues. Of these, human CDK8 contains four putative PKA phosphorylation sites at Thr-31 (in agreement with Tassan et al.), Ser-114, Thr287, and Thr-325. Murine CDK8 is potentially phosphorylated at 7 serine residues, 9 threonine residues and 3 tyrosine residues (see pages 5-6 of **Appendix B**) and CDK8 of *Drosophila melanogaster* is predicted to be phosphorylated at 7 serine residues and 6 threonine residues (see pages 1-2 of **Appendix B**).

The Examiner has stated on page 14 of the Office Action that the applicants have not provided information about specific peptides containing specific, synthetically phosphate-labelled residues that could be injected to generate an antibody response. However, phospho-specific antibodies could be made by against phosphorylated CDK8 by designing minimal

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phosphopeptides identified by the NetPhos 2.0 server and injecting such phosphopeptides into animals to generate an antibody response. It is within the ordinary skill of one in the art to identify which of the predicted sties are phosphorylated by means of phosphopeptide analysis. Antibodies directed against non-phosphorylated CDK8 are commercially available (*e.g.*, C-19 antibody from Santa Cruz Biotechnology, Inc.).

PAK6

Regarding PAK6, the Examiner, states that the relevance of phosphorylation events (by PAK6), where and when they occur, as well as how they can be related to overall cell activation are not known.

The applicants note that PAK6 is activated by phosphorylation by p38 MAP kinase and MAP kinase Kinas 6 (MKK6) (Kaur R. et al. 2005 *J Biol Chem* **280**:3323-3330). Referring to the abstract, lines 13-14, Kaur et al. states "Mutation of a consensus p38 MAP kinase target site at serine 165 decreased PAK6 kinase activity." Referring to lines 14-18 of the abstract, Kaur et al. reports that "Moreover, PAK6 was directly activated by MKK6, and mutation of tyrosine 566 in a consensus MKK6 site (threonine-proline-tyrosine, TPY) in the activation loop of the PAK6 kinase domain prevented activation by MKK6". PAK6 was first identified as an androgen receptor (AR)-interacting protein able to inhibit AR-mediated transcriptional responses.

In addition, investigators have shown that PAK6 inhibits androgen receptor signaling by inhibiting nuclear translocation of stimulated androgen receptor (Schrantz, N. et al. 2004 *J Biol Chem* **279**:1922-1931; Lee, S.R. et al. 2002 *Molec Endocrinol* **16**:85-99). Thus, the activation state of a cell is related to the level of active, phosphorylated PAK6. Since the phosphorylation state of PAK6 can be measured and it is related to the activation state of a cell, claims 1 and 15 are enabled with respect to evaluating the activation state of cells by quantifying the level of phosphorylation of PAK6.

In view of the foregoing discussion regarding CDK8 and PAK6, the applicants respectfully request removal of the rejection of claims 1 and 15 under 35 U.S.C 112, first paragraph.

Rejection under 35 U.S.C 102(b)

The Examiner has rejected claims 1, 4, 7, 8, 12, and 14 as being anticipated by Schooler et al. Claims 1 and 22 are amended to specify measurement of at least 3 specific cellular proteins. Since Schooler et al. only measures the phosphorylation level of one protein (i.e., Epidermal Growth Factor Receptor), the reference does not anticipate claims 1, 4, 7, 8, 12 and 14. Applicants respectfully request removal of the rejection.

Rejections under 35 U.S.C 103(a)

1. Schooler et al. in view of Paweltz et al.

The Examiner has rejected claims 1, 4, 7-9, 11, 12, 14, 22-27 and 29 as being obvious in light of Schooler in view of Paweltz et al. The Examiner has stated that Paweltz et al. teach that observing the phosphorylation state of multiple proteins in parallel yields a signature of diseased tissue. In the Paweltz reference, the phosphorylation state of two proteins was measured.

Appendix C is a second Rule 132 Declaration by Dr. Jose Remacle that provides an example of how the measurement of at least three proteins provides unexpected benefits of additional details regarding the contributions of specific signal transduction pathways. Referring to Exhibit 3 of the declaration, analysis of one protein (e.g., c-Jun) would have led one to conclude that the cells were not activated. Dr. Remacle states on page 2, lines 15-17:

“As illustrated in the accompanying Exhibits, one cannot predict an activation state of cells if the phosphorylation level of only one TF is analyzed. As shown in Exhibit 3, analyzing the phosphorylation of c-Jun only would suggest no difference between the two stimulation times.”

In general, determination of the phosphorylation state of only one protein does not allow prediction of the activation state of cells because intracellular pathways leading to phosphorylation of proteins can parallel each other, giving either synergistic or antagonistic effects. Thus, it is necessary to assess the phosphorylation state of more than one protein to determine the activation state of cells.

Referring to the second declaration by Jose Remacle (**Appendix C**), analysis of two proteins (i.e., Elk-1 and ATF-2) showed that either the p38 and/or JNK cascades are activated, but it was not possible to determine if one or both pathways were involved based only on analysis of these two proteins. Dr. Remacle states on page 2, lines 21-24:

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“Since Elk-1 can be activated by three cascades, namely the ERK1/2, JNK and p38 cascades, it is not possible, based on this sole result, to determine which cascade(s) is (are) activated. The dual analysis of ELK-1 and ATF-2 phosphorylation shows that at least the p38 and/or JNK cascades are activated, but this analysis still cannot answer the question.”

On the other hand, by analyzing a third protein (*i.e.*, c-Jun), it was possible to determine that increased levels of phosphorylated Elk-1 and ATF-2 were due to activation of the p38 pathway since the lack of c-Jun phosphorylation indicated that the JNK pathway was not activated. Dr. Remacle states on page 2, lines 24-25:

“The triple analysis of the Elk-1, ATF-2 and c-Jun phosphorylation shows that the JNK cascade is not activated”

Therefore, measurement of at least three proteins provides the unexpected benefit of additional details regarding the contributions of specific signal transduction pathways. In view of such unexpected advantages, the claims are not obvious in light of Schooler et al. in view of Paweletz et al. For this reason, claims 1, 4, 7-9, 11, 12, 14, 22-27 and 29 are not obvious in light of the cited references and are in compliance with 35 U.S.C. §103(a).

2. Schooler et al. in view of Paweletz et al. and Lee et al.

The Examiner has rejected claims 1, 4, 7-9, 11, 12, 14, 22-27 and 29 as being obvious in light of Schooler in view of Paweletz et al and in view of Lee et al. The unexpected advantages of measuring at least three proteins explained above are also applicable to this rejection since neither Schooler et al., Paweletz et al. or Lee et al. disclose measuring at least three proteins. Thus, the claims are not obvious and are in compliance with 35 U.S.C. §103(a).

3. Schooler et al. in view of Paweletz et al. and in view of Gustafson et al.

The Examiner has rejected claims 1, 4, 7-9, 11, 12, 14, 22-27 and 29 as being obvious in light of Schooler in view of Paweletz et al and in view of Gustafson et al. The unexpected advantages of measuring at least three proteins explained above are also applicable to this rejection since neither Schooler et al., Paweletz et al. or Gustafson et al. disclose measuring at least three proteins. Thus, the claims are not obvious and are in compliance with 35 U.S.C. §103(a).

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CONCLUSION

In view of Applicants' amendments to the Claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns that might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: June 8, 2007

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